Fanconi Anemia

Adult Head and Neck Cancer and Hematopoietic Mosaicism

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Fanconi anemia (FA) is an autosomal recessive DNA repair disorder with a very high risk of cancer.[1,2] While most of the homozygotes are identified clinically because of characteristic birth defects and early-onset aplastic anemia,[3] a subset of patients, often with milder physical and hematologic phenotypes, remain undiagnosed. They are at very high risk of neoplasms, including acute myeloid leukemias and solid tumors.[4] All types of solid tumors (combined) develop at a rate that is 48 times greater than that experienced by the general population, and the cancer hazard rate is 2% per year by the age of 24 years, with a cumulative incidence in a competing risk model of 29% by the age of 45 years.[5]

The majority of the solid tumors in patients with FA arise in the aerodigestive or gynecologic areas.[3,6] The relative risk of head and neck squamous cell carcinoma (HNSCC) in patients with FA who have not undergone bone marrow transplantation is more than 700 times greater than that of the comparable general population, and the risk of esophageal cancer is increased more than 2000-fold.[5] Fanconi anemia–associated HNSCC differs from that observed in the sporadic setting, because patients with FA are atypically young (<50 years of age) and are usually female nonsmokers and nondrinkers. In contrast, the average age at diagnosis for patients with sporadic HNSCC is in the late 60s, and the majority of such persons are men who abuse both tobacco and alcohol.

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In more than 20% of the patients with FA who were described in the literature as having developed solid tumors, the diagnosis of FA was only made *after* the appearance of their cancer.[1] Although the physical features of FA in these patients were mild, they were recognizable by clinicians with an appropriate index of suspicion. While only a few of those patients had histories of unexplained bone marrow failure, in many instances hematologic problems were either unrecognized or ascribed to adverse effects from the management of their malignancy with irradiation or chemotherapy; some patients did not have hematologic problems at all.

While the median age at diagnosis of FA is 7 years (age range, birth to >50 years), 10% of the patients described were at least 16 years of age.[3] Fanconi anemia may be underdiagnosed in older patients because (1) FA is considered to be a "childhood disease" and therefore the responsibility of pediatricians; (2) physicians who deal with adult patients do not include FA in their differential diagnoses because they have no experience with FA; and (3) there is variable expression of the physical and laboratory features of FA, and the older patients tend to have milder phenotypes. Patients with FA who are more severely affected have a much greater risk of not surviving childhood.

However, a more complicated explanation can be offered for a few of the older patients, including some in whom cancer precedes the diagnosis of FA, ie, hematopoietic somatic mosaicism.[7,8] In these cases, a random somatic genetic event occurs in

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a hematopoietic stem cell, and 1 of the 2 abnormal *FA* alleles is returned to its normal wild-type configuration, leading to correction of the *FA* defect. One abnormal copy of the *FA* gene is insufficient to cause bone marrow failure. The genetic mechanism by which the mutated allele is restored to its normal state involves mitotic recombination either because of an intragenic crossover between maternal and paternal *FA* mutations or because of gene conversion with the loss of 1 pathogenic mutation. The progeny of the gene-corrected hematopoietic stem cell have a selective growth advantage over the stem cells with 2 mutant *FA* alleles; therefore, patients in whom gene correction has occurred may have normal blood cell counts. However, the remaining body tissues still carry both mutated copies of the *FA* gene, and they therefore remain at risk of FA-related neoplastic events.

Because patients with FA may experience potentially lethal toxic effects from irradiation and chemotherapy, it is critical that patients with cancer who have FA be identified before the initiation of such treatments.[9] In general, management in such cases must be primarily surgical or based on pharmacologic modalities that do not lead to DNA damage, especially damage caused by cross-linking agents such as cisplatin, cyclophosphamide, and mitomycin. Thus, it is imperative that the possibility of FA be considered in apparently normal patients who present with the types of cancer seen in FA, including head and neck and esophageal SCCs, particularly when the patient is atypically young (<50 years of age) and does not report exposure to the usual HNSCC risk factors, ie, tobacco and alcohol. The proportion of patients with atypical clinical presentations of HNSCC that can be explained on the basis of clinically unrecognized FA is unknown, but it is certainly larger than we currently recognize.

The clinical challenges related to a delayed diagnosis of FA in HNSCC are illustrated by the patient described herein, in whom FA was recognized several years *after* treatment of her tongue cancer. We identified the molecular basis for hematopoietic somatic mosaicism, which may have permitted her bone marrow to tolerate radiotherapy, while simultaneously producing severe local toxic effects within the radiation treatment field.

REPORT OF A CASE

A 30-year-old woman presented with SCC at the base of the tongue. Radiation therapy consisted of 70.2 Gy in 1.8-Gy fractions over 8 weeks and was complicated by profound mucositis and dehydration (requiring placement of a feeding gastrostomy), xerostomia, fever, and extensive loss of teeth. A radical neck dissection was performed subsequently. At the age of 33 years, the patient developed multiple invasive SCCs on the skin of the left ear and right side of the neck (all within the prior radiation treatment field), which led to the suggestion that she might have a defect in DNA repair. Her Internet search led to her referral to the National Cancer Institute, Bethesda, Md, at which time the diagnosis of FA was suspected clinically. Her height was 145 cm and her weight was 36 kg. She had cafe au lait spots, excessive tanning, premature ovarian failure, and deafness that required hearing aids. Her face was narrow, head and eyes were small, and ear canals were narrow (**Figure 1**). Both kidneys were small on renal ultrasound, and skeletal survey identified asymmetry of the first ribs and changes in cervical and thoracic vertebrae consistent with a Klippel-Feil deformity. Three years after the diagnosis of her HNSCC, the patient had a normal complete blood cell count. Laboratory tests revealed the following values: hemoglobin, 12.8 g/dL; mean cell volume, 92.2 fL; white blood cell count, 3.7×10^9/µL; absolute neutrophil count, 2.2×10^9/µL; platelet count, 171×10^3/µL; hemoglobin F fraction, 0.6%; and serum erythropoietin, 7.6 mU/mL; these normal values indicate that there was no evidence of bone marrow suppression or stress erythropoiesis.

METHODS

All studies were approved by the institutional review board of the National Cancer Institute, and the patient signed an approved consent form. Chromosome breakage was examined in cultures of peripheral blood lymphocytes and skin fibroblasts, with the addition of 40 and 100 ng/mL of mitomycin for 72 hours or 100 ng/mL of diepoxybutane for the last 48 hours of a 72-hour culture. Colcemid was added at the end of the culture period to arrest cells in metaphase. Fifty cells were scored for the number of cells with chromosomal aberrations, the number of breaks per cell, and the number of cells with radial figures.[10-12] Mutation testing of *FA* genes was performed by high-throughput screening, using multiplex ligation-dependent probe amplification, automated denaturing high-performance liquid chromatography screening, and direct sequencing of aberrant fragments.[13,14]

The medical literature was searched for all reports of *Fanconi Anemia and cancer*, using MEDLINE and Web of Science. Information from the reports was entered into a spreadsheet (Lotus 1-2-3 Release 5; Lotus Development Corp, Cambridge, Mass), and statistical analyses were performed with a commercially available software package.[15] Sex ratios were compared with a Fisher exact test, and ages with a *t* test.

RESULTS

The diagnostic "gold standard" for FA is the chromosome breakage test, in which nearly all cultured cells show evidence of clastogen-induced chromosomal damage. A sample of the patient's peripheral blood lymphocytes was cultured with diepoxybutane or mitomycin; while some evidence of chromosome aberrations was noted, more than 50% of the cells were resistant to DNA cross-link–induced chromosomal breakage. This pattern suggested the presence of 2 populations of cells in the peripheral blood, one carrying the complete FA defect, and the other seemingly normal. This phenomenon is consistent with the presence of hematopoietic mosaicism. By way of confirmation, the patient's cultured skin fibroblasts were completely sensitive to diepoxybutane and mitomycin, thus confirming the diagnosis of FA. Her bone marrow sample was hypocellular (approximately 25%), with normal morphologic features and occasional micromegakaryocytes; there was no cytogenetically abnormal clone.

Figure 1. Patient with Fanconi anemia and tongue cancer. A, Patient at diagnosis of Fanconi anemia 3 years after diagnosis of head and neck squamous cell carcinoma. Note the short stature. B, Close-up of patient in Figure 1A. Note microcephaly, microphthalmia, and small chin.

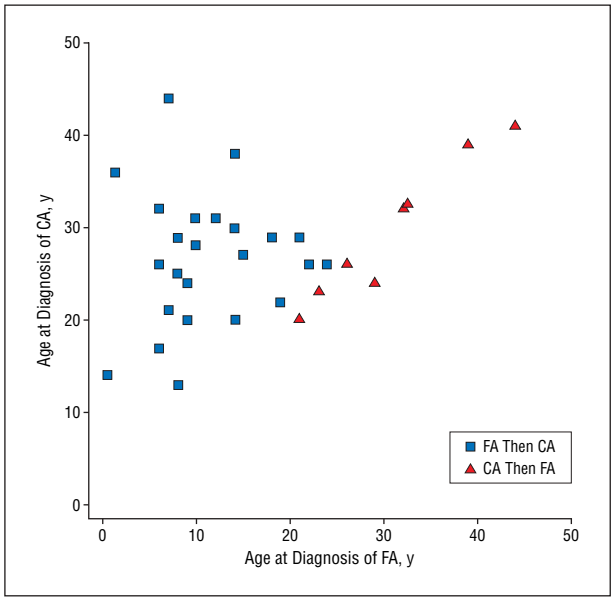

Figure 2. Age at diagnosis of Fanconi anemia (FA) and of cancer (CA). The red triangles represent 8 patients in whom the diagnosis of FA was made after CA developed. The blue squares represent 25 of the 30 patients in whom the diagnosis of FA preceded CA. Age at diagnosis of FA was not stated for 5 patients, and 2 patients were 7 years old at the diagnosis of FA and 21 years old at the diagnosis of CA.

Sequencing of fibroblast DNA detected compound heterozygosity for 2 different truncating mutations in the *FANCA* (FA group A) gene. There was a point mutation in exon 8 (790 C to T), which resulted in a stop codon (Gln264Stop). The second mutation was a 2–base pair deletion in exon 27 (2585delCT), leading to a frameshift (Cys846fxX20). DNA derived from Epstein-Barr virus–transformed lymphoblasts had only the exon 8 mutation. The most likely explanation is that there had been a gene conversion event, with loss of the frameshift 2585delCT in the cross-link–resistant lymphoblasts. The presumption is that this gene conversion occurred in a hematopoietic stem cell whose progeny had a selective growth advantage, leading to normal blood cell counts.[7]

Our previous studies indicated that head and neck cancer is the most common solid tumor in patients with FA.[1] To evaluate the relative contribution of patients in whom the diagnosis of FA was made only after the development of cancer, all reported cases of FA with aerodigestive cancer were analyzed. Cancers of the tongue, esophagus, and gingiva were the most frequent (**Table**). There were 30 patients, with 33 cancers, in whom the diagnosis of FA preceded the development of cancer, while the cancer diagnosis was made first in 8 patients (27%). Female patients outnumbered male patients in both categories (16:14 vs 7:1); the excess of female patients above the expected 50% in those in whom the diagnosis of FA was delayed is statistically significant ($P=.04$).

Important insights can be derived from the comparison of the ages for the respective diagnoses of FA and cancer (**Figure 2**). The median age at diagnosis of FA in patients in whom the diagnosis of FA preceded the diagnosis of cancer was 9.0 years (range, 0.5-24.0 years), significantly younger than the age at diagnosis of FA in those in whom cancer came first (30.5 years [range, 21-44 years]; $P<.001$). However, the respective ages at which cancer was

Table. Types of Aerodigestive Cancer in Patients With Fanconi Anemia (FA)*

	FA Before Cancer		Cancer Before FA	
Cancer Site	Males	Females	Males	Females
Esophagus	2	5	0	3
Tongue	4	7	1	1
Gingiva	4	1	0	1
Pyriform sinus	1	0	0	1
Lip	0	0	0	1
Palate	0	0	0	1
Mandible	0	1	0	0
Cricoid	0	1	0	0
Tonsil	0	1	0	0
Larynx	2	1	0	0
Oropharynx	2	0	0	0
Nasopharyx	1	0	0	0
Total No. of patients	14	16	1	7
Total No. of cancers	16	17	1	8

*There were 42 cancers reported in 38 patients. One male patient had gingival and tongue cancers, and 1 had laryngeal and nasopharyngeal cancers. One female patient had gingival and esophageal cancers, and 1 had cancers of the lip and palate. The esophageal cancers included 1 upper, 5 middle, 2 distal, and 2 location not stated. References updated from those provided in reference 1.

diagnosed in the 2 groups were similar (median ages, 26.0 years [range, 13-44 years] and 29 years [range, 20-41]; $P = .23$).

COMMENT

Appropriate identification of underlying genetic syndromes in patients with atypical HNSCC has both clinical and scientific value. Management of cancer in a patient with a DNA repair disorder, such as FA, is complicated by severe sensitivity to most forms of chemotherapy, especially regimens that include polyfunctional alkylating agents, such as cisplatin. Many patients with FA are also sensitive to radiation therapy, as demonstrated in our case.[9] An attempt to apply aggressive, multimodality treatments for HNSCC, such as surgery followed by cisplatin and radiotherapy, would probably have been lethal to our patient.[16,17] Early surgical intervention alone, with clear margins, would be the safest treatment approach for a patient with FA and newly diagnosed HNSCC. However, high risks of local recurrence and of additional primary tumors in the same region (because of so-called field cancerization) mandate close and frequent follow-up, with additional surgery as needed. At present, there are no data to indicate whether reduced doses of radiotherapy or chemotherapy could avoid severe toxic effects, while maintaining the therapeutic efficacy of full-dose treatment.

The rare association between HNSCC and FA may provide an opportunity for understanding the causal pathway of head and neck cancers in the general population. While a history of smoking and drinking is frequent in the latter setting, it is less common in patients with FA and these cancers. On the other hand, approximately 25% of sporadic HNSCC is associated with human papillomavirus (HPV).[18] The molecular progression of head and neck cancer includes inactivation of tumor suppressor genes such as *p16/CDKN2A*, *TP53*, *PTEN*, and *Rb1*, as well as activation of protooncogenes such as *cyclin D1*, *p63*, and *epidermal growth factor receptor*.[19] Tumors in which HPV is not present may have mutant *p53*, while tumors associated with HPV have wild-type *p53* alleles, since HPV E6 protein inactivates p53 protein by inducing its degradation.[20]

An epigenetic mechanism for the transcriptional inactivation of tumor suppressor genes involves hypermethylation of CpG islands. In one study, methylation was found in more than 50% of head and neck tumors, in one or more of the tumor suppressor gene *p16 (CDKN2A)*, the DNA repair gene *MGMT* (*O*^{6}*-methylguanine-DNA-methyltransferase*), or the putative metastasis suppressor gene *DAP-K* (*death-associated protein kinase*).[21]

Investigation of HNSCC in FA detected HPV DNA in 84% of the FA tumors compared with 36% of control specimens.[22,23] Oral SCCs from 2 other patients with FA were found to have chromosomal gains and losses that were similar to those seen in non-FA tumors, leading to the suggestions that the cancer pathways were similar in FA and non-FA and that FA genes might have a caretaker function in protecting against sporadic oral carcinogenesis.[24]

Support for the role of *FA* genes in the pathogenesis of sporadic cancer was provided by the recent demonstration of epigenetic inactivation of the *FANCF* (FA group F) gene (by somatic promoter methylation) in 15% of sporadic cases of HNSCC and an inverse correlation between methylation and smoking.[25] These tumors had undergone epigenetic events that resulted in the inactivation of the *FA/BRCA* pathway and thus had developed a DNA repair defect that strongly resembles that seen in patients with FA and germline mutations in the same genes.

The diagnosis of FA must be considered for all patients who have clinically atypical presentations of cancers known to be associated with this rare genetic syndrome. Seeking evidence of bone marrow dysfunction and/or the various phenotypic abnormalities that characterize FA might provide supportive evidence for that being the underlying disorder. However, our case illustrates that the presence of a normal blood cell count does not exclude the possibility that the patient might, nonetheless, have FA. Hematopoietic mosaicism (in which the blood cells do not carry the necessary 2 mutated *FA* alleles, while other somatic tissues do) can explain this confusing pattern, which has been observed by others as well as ourselves, in the context of cancer preceding recognition of FA. The first such case to be reported had 69% T lymphocytes that were resistant to cross-link damage, but the patient died of lung cancer before molecular testing.[7] In the second case, cross-link–sensitive skin fibroblasts were corrected with an *FANCA* retrovirus; a pathogenic 4–base pair deletion (1111delTGGT) was found in exon 13 of one allele, but the mutation in the other allele could not be found.[8] In our case, we were able to identify the mutations in both alleles and to document in Epstein-Barr virus–transformed lymphoblasts (for the first time) the molecular event that led to somatic correction in one allele. Although the extent of mosaicism was approximately 50% in the analysis of chromosome breakage in T lymphocytes, it was complete in the Epstein-Barr virus–transformed lymphocytes, because the gene-corrected clone presumably outgrew any uncorrected B-cell clones.

It is clear that "clinically atypical" (young female nonsmoker, nondrinker), apparently sporadic head and neck and esophageal cancer may occur in patients who have reached adulthood with undiagnosed FA. Their physical appearance may be less severely abnormal than that of patients with classic FA, and the usual clinical laboratory test results may be normal as well. We have demonstrated that somatic molecular events may lead to corrective genetic reversion in hematopoietic stem cells, while other (nonhematopoietic) tissues (in this case, presumably the cells at the base of the tongue) contain pathogenic, biallelic germline *FA* mutations. A high index of suspicion of FA is required in patients with atypical presentations of FA-associated malignancies (eg, head and neck and esophageal or gynecologic SCC or leukemia). These patients may avoid severe, potentially lethal toxic effects from their cancer treatment if the correct diagnosis is made before therapy is initiated, and they

remain at risk of other FA-related neoplastic complications, including late-onset severe aplastic anemia, myelodysplastic syndrome, and acute leukemia, as well as other solid tumors.[3] Chromosome breakage test results may be normal in samples of peripheral blood lymphocytes, and skin fibroblast tests may be required. Therefore, recognition of the underlying cancer susceptibility syndrome has profound treatment and prognostic consequences for the affected patient.

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